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Review

Innate immune responses in central nervous system inflammation

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ABSTRACT

In autoimmune diseases of the central nervous system (CNS), innate glial cell responses play a key role in determining the outcome of leukocyte infiltration. Access of leukocytes is controlled via complex interactions with glial components of the blood–brain barrier that include angiotensin II receptors on astrocytes and immunoregulatory mediators such as Type I interferons which regulate cellular traffic. Myeloid cells at the blood–brain barrier present antigen to T cells and influence cytokine effector function. Myelin-specific T cells interact with microglia and promote differentiation of oligodendrocyte precursor cells in response to axonal injury. These innate responses offer potential targets for immunomodulatory therapy.

1. Introduction

Inflammation in the central nervous system (CNS) is a defining feature of multiple sclerosis (MS) and is thought to play a role in neurodegenerative diseases such as Alzheimer's Disease. The innate signals that control CNS inflammation are of particular interest. Even in autoimmune disease where the response initiates outside the tissue or organ and the immune system may be perceived as an invader, the target tissue has capacity to respond and to participate by regulating the immune response. Glial cells, specifically microglia and astrocytes, can induce, regulate and are themselves regulated by inflammatory immune responses within the CNS. We here review central features of innate immunity and recent work from our labs that have identified novel pathways by which glial response can contribute to CNS inflammation and potentially influence regenerative responses in the CNS.

2. Multiple sclerosis spectrum diseases

Multiple sclerosis (MS) is an inflammatory demyelinating disease that predominantly affects young adult females [1]. MS has a very high prevalence in Europe, especially in northern countries. The etiology is believed to involve an infectious or other environmental trigger in genetically susceptible individuals [1]. MS typically presents as a relapsing-remitting disease, which is amenable to immune-targeted therapies, though varying between individuals and treatments, then progresses to secondary progressive MS, against which immune-directed therapies are ineffective [1]. The inflammatory pathology of MS suggests either a T cell + macrophage or antibody + complement attack on myelin and underlying axons [2]. The specificity of infiltrating T cells includes reactivity to myelin proteins. Both $CD4^+$ and $CD8^+$ T cells are implicated in MS [1,3].

Entry of T cells to the CNS involves a complex of interactions that can loosely be described as 'crossing the blood–brain barrier (BBB)'. This is described in greater detail elsewhere [4]. Aspects that are of particular relevance here include that following chemokine and adhesion molecule driven transmigration of T cells across the vascular endothelium and its associated basement membrane, these T cells interact with macrophages and dendritic cells (DCs) in the perivascular space, the fluid contents of which being ultimately contiguous with the subpial and subarachnoid compartments (discussed in [4]). The interaction with myeloid cells in perivascular

Abbreviations: AT1, angiotensin II receptor-1; CNS, central nervous system; DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; IFN, interferon; IFNAR, interferon receptor; IRF, interferon regulatory factor; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis

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space can include T cell recognition of MHC-associated myelin antigenic peptides on DCs or macrophages. From this interim compartment T cells enter the CNS parenchyma via a chemokine and matrix metalloproteinase-dependent migration across the glia limitans, another basement membrane-associated structure that is primarily composed of astrocyte end-feet [4]. Astrocytes are a prominent source of many of the chemokines that regulate immune cell entry to the CNS and for this and their participation in the glia limitans they are recognized as key elements in controlling the integrity of the BBB. Astrocytes thus play a vital role in regulating CNS inflammation. This is exemplified by clinical consequences of experimental astrocyte loss or disabling in experimental autoimmune encephalomyelitis (EAE) [5,6].

The most widely-used animal model for MS is EAE, usually generated by immunization of mice with myelin proteins or peptides. In most models, EAE is induced by $CD4^+$ Th1 (interferon-gamma (IFNγ)-producing) or Th17 (IL-17 producing) T cells [3]. In C57BL/6 mice EAE can be induced by immunization with myelin oligodendrocyte glycoprotein (MOG) or a p35–55 peptide. T cells of other specificities are recruited to CNS infiltrates as disease progresses [3]. Antibodies against MOG and other myelin antigens can promote demyelination in MS and EAE [2,3].

Innate contributions to CNS inflammation in MS and EAE are well-recognized. In the absence of microglial response inflammation does not occur where as absence of reactive astrocytes exacerbates disease [5,7]. Interestingly the disparate effects of loss of these two glial cell types may both reflect participation in events at the BBB, a simplistic generalization being that reactive microglia facilitate whereas reactive astrocytes regulate leukocyte entry. Of particular interest are findings that suggest that reactive astrocytes may selectively influence macrophage versus T cell infiltration [6]. However, microglia have other pro-inflammatory roles and are implicated in both antigen presentation to T cells (see below) and release of pro-inflammatory mediators [8]. The latter activity can be induced by stimulation through innate receptors among which the Toll-like receptors (TLRs) have received much attention [9–12]. Ligands for such responses may include viruses, as are thought to be implicated in causation of MS, pathogen-derived products such as are contained in adjuvants in EAE, and endogenous ligands that have been proposed as a consequence of tissue damage or inflammation [12].

The first approved therapy for MS were drugs based on the cytokine IFNβ, and this remains a mainstay of clinical management, especially in relapsing-remitting MS. Efficacy varies between patients depending on factors that include generation of neutralizing antibodies and underlying cytokine status [1,3,13]. The predominant mechanism of action of IFNβ as an MS therapy is thought to be reduction of cell traffic to the CNS, and there may also be effects on regulatory cytokine production [1]. IFNβ and the multi-gene IFNα family comprise the Type I interferons (IFNI) which are implicated in Toll-like receptor-driven innate responses, notably to viral infection. The latter identifies a plausible link to MS etiology. The fact that IFNI are implicated in induction of inflammatory, e.g., antiviral immune responses, and are expressed in blood and CNS of MS patients [19], poses a conundrum for how IFNβ can be effective as a therapy against MS. One suggestion has been that whereas IFNα acts systemically to promote autoimmunity, IFNβ acts locally to suppress inflammation, possibly via regulation of tumor necrosis factor (see [19]). IFNI signal through a common IFNI receptor, IFNAR, a heterodimer of IFNAR1 and IFNAR2, which signals via Jak1/Tyk2 for STAT1/2 activation. Although differential signaling outcomes have been described for IFNα and IFNβ, there is considerable overlap [19]. Mice that lack IFNAR or IFNβ show exacerbated EAE and increased leukocyte infiltration to the CNS [14–16], and IFNAR expression by myeloid cells has been shown to be critical in this regulation [14]. Considering mechanism, it has been shown that whether IFNβ alleviates EAE depends on whether IFNγ and Th1 T cells are present, whereas in Th17 EAE as well as in relapsing-remitting MS with high IL-17 serum titers, IFNβ was ineffective [17]. Our own studies suggest that IFNI signaling modulated leukocyte infiltration in response to axonal lesion in which IFNγ is not easily detected (see below) [18]. Whether these or other cytokine-mediated effects contribute to control of leukocyte traffic, e.g., via regulation of adhesion interactions, another likely component of the mechanism of IFNβ effects in MS and EAE, and how and whether the induction of immune responses in the first place might be modulated by IFNβ in MS rheumatoid arthritis and other autoimmune diseases are reviewed elsewhere [19]. As discussed below, results from our studies point to a role for IFNI-induced glial chemokines.

3. Axonal lesion as a model for innate response in the CNS

Our general approach has been to use sterile axotomy as a trigger for innate glial response, and then compare findings to those in bona fide autoimmune situations, and in combinations of the two. This has allowed us to study mechanisms underlying immune-initiated glial responses in CNS demyelinating disease, by applying autoreactive T cells as additional triggers of glial response, such as in EAE (Fig. 1). Our research has shown a role for IFNI signaling in regulating innate glial response, as well as identifying signaling pathways for astrocytes that regulate immune access to the CNS. Our findings show roles for CNS glia in regulating endogenous innate cytokine and chemokine production, which in turn regulate immune cell entry.

3.1. Cellular sources of innate cytokines in the CNS

We have shown that microglia upregulate interferon response factor-7 (IRF7) in response to sterile axotomy and in EAE [18] (Salem, Khorooshi and Owens, unpublished). This implicates microglia as a source of IFNI, a point of intuitive acceptance that nonetheless remains to be rigorously demonstrated. Although IFNβ was detected by ELISA in the CNS of mice with EAE by Prinz et al. [14], it is more commonly detected via surrogate markers such as IRF7. This is true even in CNS virus infections, and reflects the biological potency of IFNI as an innate mediator [20]. The fact that interferon response factors are implicated both in actual response as well as feedforward induction of the cytokine itself means that interpretation of such findings must always be nuanced. All cells of the body are considered to have the capability to elicit an IFNI response, e.g., in innate receptor response to viral infection. However, we do not find that innate-responsive astrocytes express detectable IRF7 in response to axonal lesion (see below). Myeloid cells, especially DC, are favored candidate cell sources for the induction of immune regulatory cytokine levels [3,21].

We have shown that mice lacking IRF7 develop more severe EAE, with increased leukocyte infiltration to CNS (Salem, Khorooshi and Owens, unpublished). This is broadly consistent with the published work from Issazadeh, Fish, and Prinz and their colleagues [14–16] that shows that mice which lack either IFNβ or IFNAR show exacerbated EAE and enhanced leukocyte infiltration to the CNS in EAE. Our findings in the axonal lesion model generalize this observation to a non-autoimmune injury-induced innate response that may have regenerative consequences (see below). Thus endogenous or innate IFNI production is not only induced by pathogen-mimicking adjuvant-based immunization, but also in response to sterile injury in the CNS.

Prinz et al. used Cre-lox transgenic systems to show that myeloid cells were key responders to IFNI for regulation of EAE [14]. Taken together with results from adoptive transfers, which indicated

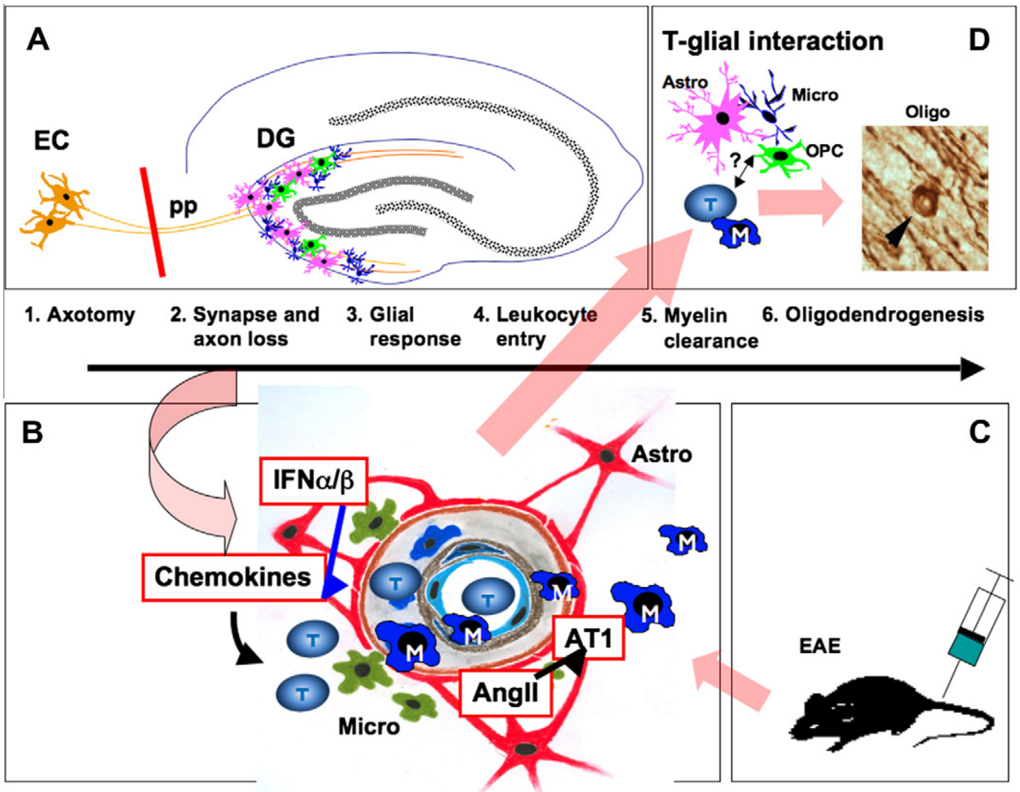

Fig. 1. Schematic to illustrate points referred to in this article. Panel A depicts lesion of the perforant pathway (pp) axons leading from entorhinal cortex (EC) to the dentate gyrus (DG) of the hippocampal formation. Synapse loss resulting from axotomy leads to activation of microglia (blue) and astrocytes (purple) in the outer molecular layer of the DG. Oligodendrocyte precursor cells (OPC, green) are also present in the DG. The glial response leads via chemokine production to leukocyte entry, which is depicted in Panel B. T cells and macrophages cross the vascular endothelium in the post-capillary venules to enter perivascular space and then cross the astrocytic (Astro) glia limitans to enter the CNS parenchyma, where they can interact with microglia (Micro). Interaction with myeloid cells also occurs within the perivascular space. Type I IFN (IFNα/β) produced by microglia acts on astrocytes and microglia to reduce chemokine expression which contributes to Type I IFN control of leukocyte infiltration. Macrophage infiltration is also controlled by angiotensin II (Ang II) signaling via the Ang II-receptor-1 (AT1). For simplicity, leukocyte entry during EAE (Panel C) is considered to be similarly controlled. (Panel D) T cells that enter the CNS promote myelin clearance by co-infiltrating macrophages (M) and by resident microglia (Micro). Interaction of oligodendrocyte precursor cells (OPC) with these results in differentiation to mature oligodendrocytes (Oligo).

that the key responding cell was recipient rather than donor-derived [15] and not a T cell [14], there are clear pointers that myeloid cells, perhaps within the CNS, participate in IFNI regulation of inflammation. Our findings add that CNS microglia are a likely source of IFNI and we would propose that this contributes to natural regulation of autoimmune CNS inflammation.

3.2. Regulation of non-autoimmune leukocyte infiltration by innate cytokines in the CNS

Mice lacking IFNAR show 2-fold increased glial chemokine expression and leukocyte infiltration to the hippocampus in response to sterile axotomy [18]. This complements previously-described findings in EAE [14–16]. Thus, IFNI response may regulate immune cell entry to the CNS regardless of the primary inducer or the outcome. Although this leaves unanswered the relative roles of IFNα and IFNβ, it expands our understanding of the contribution of IFNI signaling to innate CNS response. Taken together with other observations that TLR signaling controls injury-reactive leukocyte infiltration to the hippocampus (reviewed in [11]), this has general implications for autoimmune and host-protective immune responses in that some innate signals are responsible for initiating response, as in the case of a viral infection, and others may limit the extent of inflammation. Such coordination between immune system and the target tissue of response reflects highly-evolved regulation.

3.3. Antigen-presenting roles for CNS-resident myeloid cells

The ability of the tissue environment to regulate local inflammation may extend to the quality of autoreactive T cell response. It is well-established that transfer of experimentally-biased Th1 or Th17 CD4 T cells is an effective means of inducing EAE [3] and that depending on the cytokine profile of the transferred T cells, their localization within the CNS and outcome of response may differ [22,23]. But there have also been reports that the CNS itself may participate in the promotion of a Th1 response [24]. This would imply activity of CNS-resident antigen-presenting cells and perivascular DC are an obvious candidate, because of their location. Microglia may also present antigen for secondary T cell response [25] and a $CD11c^{+}$ microglial subpopulation that arises during demyelinating or inflammatory responses is of particular interest [26]. However the location of CNS resident DC at extra-parenchymal sites [21,27] makes a compelling argument that they would play a key role. Demonstration that a CNS-resident cell could influence Th1 or Th17 cytokine profiles is achieved via in vitro analysis of cells that can be isolated relatively straightforwardly [25,26]. Such approaches rely on flow cytometric phenotypes including those based on relative CD45 levels being useful indicators of the microenvironment from which the cell derived in vivo [25,26]. Direct intervention in vivo is possible via local application of replication-defective viral vectors. We have taken this approach to introduce adenovirus which express IL-18 binding protein to the intrathecal compartment of the CNS of mice immunized for EAE [28]. This protein is a natural inhibitor of IL-18 which is implicated in the induction of IFNγ and thus a Th1 response. Unexpectedly we find that adenoviral IL-18 binding protein inhibits the production of Th17-inducing cytokines when DC were infected in vitro, and correspondingly this adenovirus inhibited IL-6, IL-23, and TGFβ, Th17 responses and EAE, when administered in vivo [28]. While the precise molecular details of how IL-18 and its binding protein works remain unclear [28,29], it is striking that introduction of a viral vector encoding a regulatory protein known to affect cytokine production by DC, to the same compartment where perivascular and subarachnoid DC are found, could so convincingly switch a peripherally-immunized Th17 response to (in this case) a disease-regulatory Th1 response. The implication is that T cells are susceptible to effector modulation as they transit the perivascular space. Whether analogous effects are mediated by other antigen-presenting cells within the CNS parenchyma, as might conceivably be involved in epitope spreading [3], remains to be established.

4. Cytokine production by innate immune cells in the CNS

Recent studies have shown a related role for T cells that express a gamma–delta TCR (γ/δ cells) in EAE, notably the ability to produce IL17 in response to the caspase-1-processed cytokines IL1β or IL18 [30]. γ/δ T cells are considered an innate T cell subset, because of their limited repertoire and recognition of invariant, frequently tissue-associated antigens that is independent of antigen processing and MHC I or II association. γ/δ T cells are also a source of IFNγ. They are cytotoxic towards oligodendrocytes, and have long been known to be present in the CNS of MS patients as well as in EAE, where they have variously been reported to be pro-pathogenic and protective (reviewed in [10,31]). Now that a role has been described for this cell type in EAE as an innate source of inflammatory cytokines and thus that they bridge innate and adaptive immunity, there is even more reason to understand the precise contribution of γ/δ T cells to MS, as well as other autoimmune diseases. The fact that EAE is attenuated in mice that lack γ/δ cells (see [10,31]) confirms the importance of their role.

Another unconventional or innate T cell lineage that is implicated in regulation of inflammatory cytokines in MS and EAE is the natural killer (NK)-T cell, which share properties of NK cells (see below) and T cells. NK-T cells have a limited antigen-recognition repertoire due to expression of an invariant TCRα chain, and are particularly noted for their response to CD1d-associated lipid epitopes. Like $\gamma/8$ cells, they have been implicated both as protective and pro-pathogenic. They are a source of regulatory cytokines and also a source of IFNγ and IL17 [10].

Other innate cell types that are implicated in regulation of MS and EAE include natural killer (NK) cells and mast cells. The role of these cells has been recently reviewed [10]. Despite their cytotoxic potential, which they share with γ/δ and NKT cells, NK cells are more strongly implicated in regulation of MS and EAE, and it is thought, again like NKT and γ/δ T cells, that cytokine secretion is the primary mechanism of this effect [10]. Mast cells likewise have been suggested to have multiple roles in MS despite their obvious potential for cytoxicity via degranulation. The fact that all of these innate immune cell types can be shown to exert regulatory effects via cytokine production, in parallel with more dramatic cytotoxic activity in responses against pathogens, allows speculation that they normally play a tonic or homeostatic role in the CNS and that this is a key component of the CNS innate immune response.

Nevertheless, innate cytotoxicity likely plays an important role in pathogenesis. Microglia are implicated as a source of both reactive nitrogen and oxygen species. These mediators are key elements in anti-pathogen defences, especially when produced by peripheral macrophages. Reactive oxygen species (ROS) have been shown to mediate oligodendrocyte and neuronal cytolysis and are thus strongly implicated as effector in demyelination and axonal damage in MS [1,2]. Metalloproteases and myeloperoxidases are also produced by activated macrophages and microglia in the CNS and contribute to reactive tissue damage in MS and EAE [1,2,8,10]. Phagocytosis of degraded myelin sets up a feed-forward loop involving the adaptive immune response, as has already been discussed. It is important to keep in mind that microglia, like innate immune cells, probably normally have a homeostatic role, i.e. they were not placed in the CNS in order to cause MS. Thus, their potential for regulation of inflammation must also to considered, as will be discussed below.

5. Innate immune functions of astrocytes

The ultimate barrier to entry of leukocytes to the CNS parenchyma is the glia limitans and this focuses attention to astrocytes. Astrocytes that are activated in response to sterile axotomy do not express IRF7 but do express and phosphorylate STAT1 and STAT2 (which act as signal transducers downstream of IFNAR), and are a potent source of chemokines. NFκB-and IFNAR-signaling pathways have been shown to regulate the STAT2 and chemokine responses, respectively, and thus there is general convergence with response to IFNI [18,32]. The importance of the NFκB signaling pathway was shown in transgenic mice that express a dominant negative IκBα which can not dissociate from NFκB, selectively in astrocytes under control of a GFAP promoter [33]. Stereotactic axotomy in these mice resulted in half the normal level of CCL2 message and of locally-infiltrating leukocytes [32]. The dichotomy between this decreased leukocyte infiltration and the increase in mice lacking IFNAR poses difficulties for mechanistic linkage, other key differences being that STAT2 and CCL2 were not affected by IFNAR deficiency [32]. It is nevertheless apparent that astrocytes contribute significantly to innate chemokine responses and that such responses are regulated by microglial-derived IFNI, in the situation where response is initiated within the CNS. It is tempting to speculate that an analogous microglial-astroglial axis might operate under circumstances of immune inflammation in the CNS, where post-infiltration microglial response might then act as a brake on further immune cell entry via regulation of astrocytes at the glia limitans. The extent to which such a natural regulatory mechanism might normally act to prevent emergence of clinical pathology is unknown.

5.1. Selective control by astrocytes of macrophage versus T cell infiltration

The renin angiotensin system is well-described for its regulation of vascular tone and blood pressure under conditions of acute stress. Components of the renin-angiotensin system are also expressed in the CNS and are implicated in vascular responses, especially in stroke [34]. Given the need for vascular integrity for normal CNS functioning and the association of blood–brain barrier breakdown with MS, we and others have asked whether the renin-angiotensin system and especially angiotensin II play a role. Wosik et al. showed that human astrocytes are a source of angiotensin II that can act via angiotensin II receptor I (AT1) on endothelial cells, and that AT1 was upregulated in MS [35]. In contrast to human CNS, AT1 is expressed by astrocytes in rodents, not by endothelial cells, and we have confirmed this in mice [36]. AT1 was upregulated specifically by astrocytes in the dentate gyrus following axonal injury. We further showed that AT1 blockade by in vivo administration of the specific inhibitor candesartan led to increased influx of macrophages, but not T cells, in response to axonal lesion [36]. This suggested a role for Ang II signaling to astrocytes for maintenance of blood–brain barrier integrity. Nevertheless, we could not detect overt blood–brain barrier breakdown in candesartan-treated mice, nor is blood–brain barrier breakdown (as measured by horseradish peroxidase leakage to parenchyma) a feature of this innate response model. Our findings stand in apparent contrast to those of 2 other groups who have shown that AT1 blockade either with candesartan or with losartan led to amelioration of EAE and reduced T cell and macrophage infiltration to the CNS [37,38]. One could assume that this reflects differences between autoimmune versus innate astrocyte response. We cannot otherwise explain the discrepant results regarding AT1 and leukocyte entry but they stand as a caution to those who would exploit this receptor system for MS therapy. We also find that inactivation of astrocytes by gancyclovir treatment of GFAP/HSVtk-Tg mice exacerbated EAE, and enhanced macrophage infiltration [6]. Despite that the thymidine kinase transgene is designed to be a 'suicide' gene for cells expressing it when treated with gancyclovir, our experience matches that of Heppner et al. with microglia [7], in that astrocytes were not markedly depleted in gancyclovir-treated mice in the time frame of our experiment, nor was there a significant loss of aquaporin-4 staining [6]. Nevertheless, there was a pronounced effect on EAE with selective increase in macrophage infiltration. Exacerbation of EAE by such intervention was also shown by Voskuhl et al., who emphasized the protective aspects of the reactive astrocyte scar [5]. The fact that gancyclovir treatment was initiated at the time of onset of EAE in our study may have contributed to the selectivity for macrophage entry, given that T cells had already infiltrated the CNS. Thus by both AT1 blockade and astrocyte disabling, astrocytes at the glia limitans are thus implicated as 'gatekeepers' for the brain [36], whether under innate or autoimmune conditions, and they selectively control macrophage versus T cell entry, at least in the experimental systems that we have applied.

6. Interface between innate and adaptive immunity

It is well-accepted that the innate response sets the stage for the evolution of an adaptive response. In this regard it is of interest whether the innate response induces a pro-inflammatory or anti-inflammatory milieu, corresponding to the M1 and M2 environments that have been described for macrophage activation [39], and if the outcome of an adaptive response superimposed on an innate response is of benefit to the host. The concept that inflammatory responses are deleterious derives from pathological circumstances that may be interpreted as over-reactions on the part of the immune system, which would otherwise have been selected through evolution for host-benefit. Experimental systems based on mild injury where repair is both desirable and possible may have better potential to reveal beneficial aspects of inflammatory responses than models based on infection or infection-mimicking adjuvants, where the goal is to expel a pathogen regardless of collateral damage, or models of cerebral catastrophes such as stroke and hemorrhages, where the primary goal is the survival of the individual. These somewhat philosophical considerations receive support from recent findings, that have shown a beneficial outcome of interaction between autoimmune T cells and CNS microglia at the level of oligodendrogenesis following mild injury.

6.1. T cell infiltration to sites of innate glial response

The interface between innate and adaptive immunity in the brain is exemplified by downstream consequences of leukocyte entry induced by sterile injury in the form of stereotactic transection (axotomy) of the entorhino-dentate perforant pathway [40] (Fig. 1A). Recent findings suggest that infiltrating myelin-specific T cells stimulate microglial-macrophage responses and cytokine production [41]. Although T cells normally infiltrate the hippocampal formation following perforant pathway transection, the number of T cells infiltrating the neural parenchyma is very limited [41,42]. Numbers of T cells infiltrating the neural parenchyma, however, increased dramatically when myelin-specific CD4+ T cells were adoptively transferred to these mice prior to axotomy [41]. Such infiltration was lesion-dependent, arguing that the axonal lesion-induced astro- and microglial response induced the microvascular endothelial cells to upregulate their expression of adhesion molecules, critical for leukocyte trafficking across the BBB [4]. This points to a fast and efficient signaling by the glial cells, that initially sensed the degeneration of axons and terminals, to the endothelial cells, leading to a lesion-specific immune cell recruitment.

While cellular recruitment was at least in part due to glial expressed chemokines [43], facilitated by increased expression of adhesion molecule expression [4], the continued presence of T cells in the neural parenchyma depended on antigen specificity, in that numbers of infiltrating T cells were approximately 100-fold higher than in controls only when myelin-specific T cells had been transferred and not when ovalbumin-specific T cells were transferred [41]. It can be assumed that this reflects interaction with cells presenting myelin antigens, which would be consistent with the uptake of myelin basic protein (MBP) in microglia-macrophages [41], although the exact identity, location and molecular details of the T cell-antigen presenting cell transaction remain to be elucidated. Detection of message for Th1 cytokines and scattered IFNγ-producing cells, visualized by in situ hybridization, support that a T-cell receptor-driven activation event had occurred [44].

6.2. Microglial activation by T cells

Consequences of the elevated myelin-specific Th1 cell infiltration observed in zones with axon and synapse degeneration pointed towards a pro-regenerative role for activated T cells. Thus Th1 cell infiltration stimulated microglial cell population expansion and cytokine expression, and dramatically increased microglial-macrophage phagocytosis and clearance of myelin debris, which has been shown to be a prerequisite for successful axonal outgrowth and remyelination in other experimental systems [45]. Although the perforant pathway fibers are thinly myelinated with multiple boutons on passage [40], transected perforant pathway fibers are thought to undergo anterograde axonal (Wallerian) degeneration in the same way as heavily myelinated fiber tracts distal to sites of axonal transection in brain and spinal cord. Since the hippocampal formation is part of the cerebral cortex, the perforant pathway deafferented dentate gyrus also shares features with MS lesions of the cortical grey matter, that appears to be dominated by activated microglia. Importantly, grey matter lesions are already present in relapsing-remitting and acute MS and become more prominent in secondary progressive MS [2]. If antero- and retrograde neurodegenerative phenomena play a role in the formation of cortical lesions, studies of lesion-specific T-cell recruitment and its consequences will be relevant to understand the pathobiology of these lesions.

6.3. T cell-stimulated oligodendrogenesis

Remyelination of denuded axons is a component of the eventual functional recovery which takes place over a period of days to months [46]. It should be understood, that since the transected perforant pathway axons do not grow back into the dentate gyrus, the regenerative response in the dentate gyrus consists of collateral sprouts from other afferent fiber systems, that also trigger a myelination response [44,47]. This is consistent with other work from us, showing that lesion-induced axonal sprouting in the regio inferior hippocampus was associated with generation of new oligodendrocytes and significant increase in the length of myelinated fibers [48]. Although not known with certainty, it seems likely that myelination of sprouting axons is regulated by some of the same signals that regulate remyelination of denuded axons as occurs in plaques in the CNS of patients with MS. In both circumstances the initial step is stimulation of the ubiquitously present oligodendrocyte precursor cells to proliferate and differentiate into new oligodendrocytes [44,47]. We found that myelin-specific T cells stimulated the generation of new oligodendrocytes via differentiation of newly generated precursor cells. Whether this was due to pro-differentiative cytokines or trophic factors acting directly on oligodendrocyte precursor cells, or cytokines or trophic factors acting indirectly on them via microglia and/or neurons remains to be established. Note that although not addressed by our study, a role for astrocytes as another T-cell-driven glial source of neurotrophins cannot be excluded. We originally speculated that the observed effects were due to $CD4^+$ Th2 cells producing IL-4 acting on microglia to downregulate TNF and upregulate lesion-induced production of insulin-like growth factor-1, the latter being neuroprotective and a stimulant of oligodendrogenesis [46], or to production of oligodendrotrophic brain derived neurotrophic factor (BDNF) by these cells [49]. The observation of a predominant Th1 response in this model, however, calls attention to the fact that IFNγ-producing Th1 cells may, like Th2 cells, themselves produce BDNF [49]. The likely involvement of CNS innate microglia in the induction of these potentially protective adaptive responses reinforces that innate immunity in the CNS, although it can be destructive, is of benefit to the host if adequately regulated.

7. Concluding remarks

We have reviewed roles for innate, mostly glial, response in the CNS that direct and control entry of leukocytes to the CNS, that regulate immune responses within the CNS and which may translate an immune provocation towards a CNS-regenerative or -protective outcome. In all cases studied the findings reveal a capacity for the CNS, often considered a 'target tissue' to actively participate in interaction with the immune response.

Implications for MS include that for optimal effect of therapies which seek to modulate immune response they should have access to the CNS as well as act peripherally, since regulation of the immune response continues after parenchymal entry. There are also likely to be benefits from exploitation of intra-CNS endogenous immunoregulatory systems, the IFNI pathway representing an obvious example, that is currently mimicked by exogenous application of one of its activating ligands. These perspectives have not attempted to deal with heterogeneity within the MS spectrum of diseases, which likely contribute to some of the perceived treatment failures. However, it seems clear that tailoring of therapies to a better-understood CNS-immune dialogue will be the way of the future.

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